THE LIFE HISTORY OF A TREMATODE (LEVINSENIELLA CRUZI?) FROM THE SHORE BIRDS (LIMOSA FEDOA AND CATOPTROPHORUS SEMI-PALMATUS INORNATUS)[1]

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Introduction

Lebour (1911) has described a cercaria (*C. ubiquita*) from British snails (*Paludestrina stagnalis*, *Littorina obtusata* and *L. rudis*). She found "Cercariæ exactly corresponding with *C. ubiquita*, but without the glands, inside the tissues of *Carcinas mænas*" and similar metacercariæ were found by her in *Cancer pagurus*, the third host in the cycle being presumably the herring gull (*Larus argentatus*). The same or a closely related larva has been found by Stunkard (1932) in *Littorina rudis* and *L. littorea* at Roscoff, France, and Rees (1936) has observed similar larvæ in these snails and in *L. obtusata* at Aberystwith, England. The adult form of these larvæ is supposed to be some species of *Levinseniella*, but thus far the various steps in the life history have not been definitely established.

In a study of the trematodes of the La Jolla region on which I am engaged I have found a stylet cercaria infesting the snail *Olivella biplicata* which differs in some particulars from those of Lebour and Stunkard, and whose probable adult form I have located in the shore birds (*Limosa fedoa* and *Catoptrophorus semipalmatus inornatus*), while the metacercaria parasitizes the sand crab (*Emerita analoga*).

I will first describe the cercaria, the metacercaria and the adult worm, and then give the evidence on which my conclusions regarding the life history are based.

The Cercaria

The cercaria (Fig. 1) is one of the spineless xiphidio-cercariæ, possessing a stylet from 24 to 32 μ long, and a set of eight unicellular penetration (?) glands, but no pigment spots. In fixed specimens the body averages 165 $\times$ 46 μ and the tail 124 μ in length. It has an oral

[1] The following study has been conducted at the Scripps Institution of Oceanography, to whose directors, Dr. T. Wayland Vaughan and Dr. H. U. Sverdrup, and other members of the staff I am indebted for many courtesies.

sucker 34 μ in average diameter, but no acetabulum, or gut. The excretory bladder is bifurcate, but further details of the excretory system are not evident.

Perhaps the most interesting feature of the larva are the penetration (?) glands, which are in two sets, an anterior set of four larger, and a posterior one of four smaller glands. Their arrangement is shown in Fig. 1, where they are designated by numbers 1–8 respectively. Each gland has a duct which follows a more or less sinuous course depending on the state of contraction of the worm, and which opens at the anterior border of the sucker. Lebour states that there

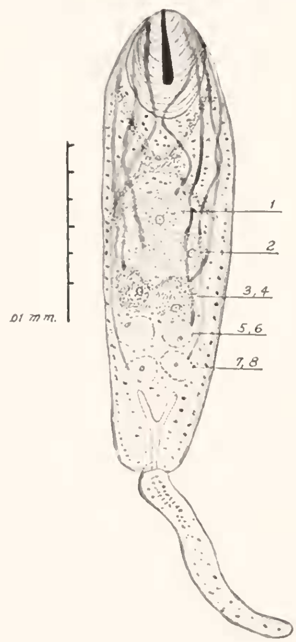

FIG. 1. Cercaria showing stylet, sucker, nerves, excretory bladder and glands (1–8). For description of latter, see text. Outline with camera, details free-hand.

are but four ducts for the group of glands in her larva, but their close apposition and sinuous course renders their individual identification difficult and might easily lead to confusion about their actual number. My specimens, when stained with neutral red, show clearly one duct for each gland. Both glands and ducts stain intensely in this stain.

These glands differ markedly; not only in size, but also in the physical and apparently chemical character of their contents. Glands No. 1 and 2 are finely granular and do not ordinarily take an eosin stain. Occasionally Nos. 1 and 2 stain lightly in eosin but much less intensely than Nos. 3 and 4. Numbers 3 and 4 are also finely granular, though the granules are perhaps a trifle coarser than in Nos. 1 and 2, but they take the eosin stain intensely. Glands 5–8 stain very lightly. They contain only a few eosinophile granules which are scattered through the meshes of a rather open reticulum, so that the whole cell appears as an open space in the sections with what is usually a pycnotic,

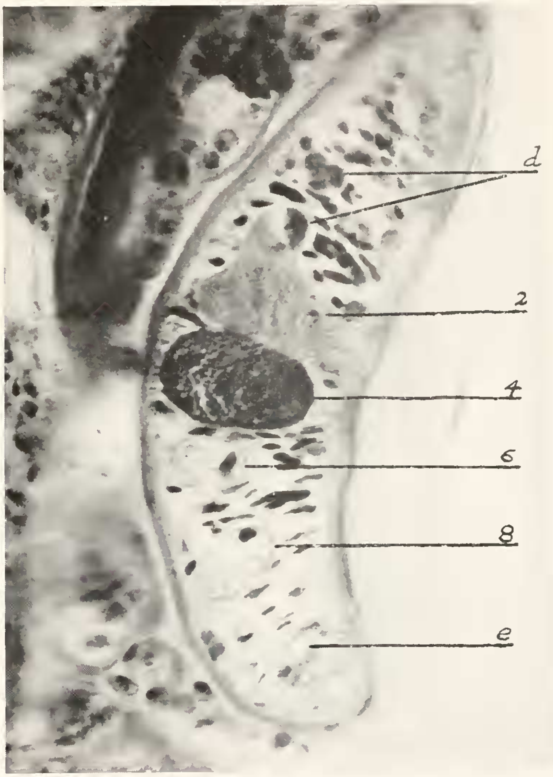

Fig. 2. Photomicrograph of long section of cercaria showing glands 2, 4, 6 and 8 (see Fig. 1 and text). *d*, ducts; *e*, excretory bladder; × 860.

heavily stained nucleus near the center. The nuclei in all eight glands, however, vary widely, sometimes being pycnotic and densely stained, and at other times vesicular and so lightly stained as to be scarcely visible. Especially is the latter true of glands 1 and 2. That these differences are real and not artificial is indicated by the fact that they may occur in neighboring larvæ in the same sporocyst. These structural and staining differences are evidence of functional differences in the two sets of cells 1–4 and 5–8 respectively. It is probable that each set elaborates a different secretion, for were the differences in structure and staining capacity due to different stages in the elaboration of the *same* secretion the two sets of cells should vary in different individuals. But this is apparently not the case; each set is distinct in every cercaria. The difference in eosinophily between cells 1, 2, and 3, 4 respectively is probably explicable by differences in the stage of development of the same secretion, but this will not explain the difference between sets 1–4 and 5–8.

Stunkard (*loc. cit.*, p. 337) says of his larva that "the cells do not stain with neutral red, but the secretory granules are clearly visible . . . (which granules) stain a deep blood red in the terminal portion of the ducts and frequently accumulate there to form enlargements," while Rees (*loc. cit.*, p. 622) says that "only the two anterior pairs (of cells) and their ducts . . . (stain) a deep red. The remaining cells and their ducts have never been observed to take up the intravital stain." All eight cells in my larva stain uniformly in neutral red, the differences appearing in the material stained with eosin.

It is difficult to harmonize these various observations with one another. They *may* indicate specific differences in the cercariæ themselves but it is not impossible that they are due to functional differences in the glands at different times and under different conditions. Possibly they are the effect of different hosts.

The infestation of the snail is considerable, 6 out of 98 specimens examined being infested.

The shedding of the cercariæ appears to be a seasonal matter, although other, as yet obscure factors may affect this. In February and March, 1935, cercariæ were easily obtained by isolating snails from the beach near the Scripps Institution, whereas from November, 1936 to January, 1937 isolation of several hundred snails produced no cercariæ. In May and June of 1937, a few cercariæ were obtained from several hundred snails, but in July and August, 1937, isolation of several hundred snails failed to produce any. That some of the apparently sterile snails were infested, however, was proven by dissection of several specimens. Lebour (*loc. cit.*) also has observed that snails are free from *C. ubiquita* in winter, while their walled cysts (metacercariæ) are present in crabs only during spring and summer and early autumn. I have not made sufficient study of this matter, however, and hence cannot define definitely the seasonal cycle of the life history, if such exists, which appears to be the case.

The Metacercaria

The cercariæ in the crab lack tails, but whether these are lost before or after entrance I cannot say. The former appears more likely, however, as the tails are frequently detached from the cercariæ while swimming. Soon after entrance the glands and ducts become broken down and are identifiable only as irregular masses which still stain in neutral red. Within two or three days the cercaria secretes a cyst within which it curls up and begins its development into the metacercaria.

This naturally varies in size with age, the older ones reaching a diameter of 0.3 mm. with a cyst wall about .025 mm. thick. It is very slightly ovoid in shape and is found in the connective tissue of the crab, chiefly among the liver tubules. Practically every crab examined, in the La Jolla region, over 6 mm. in length is infested. I have not determined the maximum number of cysts which one crab may carry, but it is probably several dozen.

The Adult

The adult worm agrees closely in all important details with the description of the genus *Levinseniella* as given by Jägerskiöld (1900). The outline is narrower in front and broader behind, and when much expanded the body may be slightly constricted just in front of the acetabulum. This latter is located about two-thirds the length of the body from the anterior end. The anterior two-thirds of the body is spinose, the spines gradually diminishing in size and disappearing at about the level of the acetabulum.

Judging from the figure (unlabeled) and the description (in Portuguese) of *L. cruzi* by Travassos (1920) my specimens belong to this species, although the difference in the orders of the hosts (*Anseres* and *Charadriformes*) and in locality (California and Rio de Janeiro, Brazil) render this uncertain. As shown in Table I, the dimensions of my specimens correspond rather well with those of *L. cruzi*,[2] the chief differences being in the size of the oral sucker, which in my specimens averages 0.061 mm. in diameter while in the former it averages from 0.092 to 0.113 mm., and in the length of the ceca (0.134 and 0.163 to 0.241, respectively).

Perhaps the most striking feature of agreement between the two is in the size of the eggs, which average larger in both than in any other species known to me. The average of 24 eggs from my specimens fixed in aceto carmine is 0.036 by 0.017 mm., while in *L. cruzi* they average from 0.021 to 0.035 by 0.012 to 0.014. When fairly extended in life my specimens range from 0.55 to 0.9 mm. in length, while *L. cruzi* ranges from 0.609 to 0.713 mm. While there thus appear to be certain minor differences between my worms and those of

[2] Presumably measured from fixed material although Travassos does not so state.

TABLE I

Table I showing average measurements in μ of fresh and preserved specimens, with the number of specimens averaged for each measurement in parentheses. With the exception of five eggs, all preserved specimens were fixed in aceto-carmine. There is some overlap in the two sets, i.e. a few specimens were measured first in the fresh, and then in the preserved condition. Column 3 shows the measurements of *L. cruzi* from Travassos (*loc. cit.*).

	Fresh	Preserved	*L. cruzi*
Length	692 (14)	676 (15)	609–713
Width	193 (14)	303 (15)	222–313
Oral sucker	61 (12)	61 (14)	92–113
Pre-pharynx	37 (3)	36 (5)	14–56
Pharynx	33×21 (8)	32×23 (9)	35×53–21×35
Post-pharynx	168 (4)	170 (10)	63–120
Ceca	112 (7)	134 (10)	163–241
Acetabulum	54 (15)	55 (15)	56–71
Acetab. distance (from ant. end)	485 (9)	457 (9)	
Penis diameter	23 (9)	23 (9)	
Seminal vesicle	67×39 (5)	67×38 (8)	
Ovary	74×49 (12)	71×54 (15)	56–92
Right testis	97×58 (12)	93×69 (14)	
Left testis	90×61 (12)	89×65 (14)	
Egg		36×17 (24)	35×14–21×12

Travassos, they are insufficient in my judgment for separating them specifically, and I am therefore, in spite of the difference in hosts and locality, provisionally assigning my specimens to his species.

The Life History

The evidence upon which my conclusions regarding the life history of this worm are based is briefly as follows:

(1) the presence in the sand crabs of stylet cercariæ, and of all stages in the developing metacercariæ from that of the cercaria surrounded by a very thin cyst, but still having the stylet and remnants of the penetration (?) glands to those of the trematode, which resembles the adult in every way save for the presence of eggs in the uterus;

(2) the experimental infestation of crabs with cercariæ;

(3) the presence in the birds of adult worms which resemble in every way the excysting metacercariæ, save for the presence of eggs in the uterus, and the presence of both encysted and excysted metacercariæ in the birds' stomachs after they have fed on sand crabs.

There is apparently in nature a universal infestation of all crabs more than 6 mm. long. I have not recorded the number of crabs which I have examined (50–100), all of which were infested. It is probable that infestation begins when the crabs are about 5 mm. long as I have found specimens of this size containing cercariæ. Until they reach a length of at least 5 mm. the crabs are less frequently infested. Of 30 specimens of this size or less which I examined in nature only 8 contained cercariæ. It is evidently a matter of age, and consequently length of time of exposure, which determines the infestation, for the larger specimens are more heavily infested than the smaller ones, and very young crabs are susceptible to experimental infestation. The season of the year is possibly also an important factor, for, as already shown,[3] the deposition of cercariæ by the snails may be dependent on the season.

I have performed three sets of experiments in an attempt to demonstrate the transfer of cercariæ from snail to crab, the results of which are summarized in Table II.

In the first of these a number of cercariæ were obtained from snails which shed them naturally, transferred to a finger bowl with ten crabs from 4 to 5 mm. long, and left for from three to four days, after which, examination of the latter showed that seven out of eight (all of which were examined at the time) contained from one to four cercariæ each while the remaining crab which was dissected 42 days later was negative.

[3] See page 322.

As a control 15 crabs collected on the beach within four weeks of this experiment gave a ratio of 5 infested to 10 non-infested specimens.

An experimental infestation of seven out of nine young crabs, while 10 of 15 in nature were uninfested, has a probability, on the basis of chance alone, of .03333.[4]

A second group of experiments consisted in holding for one or two days 18 young *Emerita* in finger bowls, together with cercariæ which had just been shed by snails. Seven of these were subsequently found to be infested. In the control for this experiment, out of 20 young crabs none were infested. In this experiment and its control the crabs were kept for several days previously in running sea water which was filtered through several inches of sand, thereby eliminating the likelihood of an accidental infestation. The probability of this result occurring on the basis of chance alone is .0021.[4] In explanation of

Table II

Showing results of experimental infestation of *Emerita* with the cercaria of *Levinseniella cruzi* (?)[5]

Series 1		Series 2		Series 3	
Experiment: *Emerita* exposed to cercariæ		Experiment: *Emerita* exposed to cercariæ		Experiment: *Emerita* exposed to *Olivella*	
Positive	Negative	Positive	Negative	Positive	Negative
7	2	7	11	17	18
Control: *Emerita* collected on beach		Control: *Emerita* in filtered sea water		Control: *Emerita* in unfiltered sea water	
Positive	Negative	Positive	Negative	Positive	Negative
5	10	0	20	6	40

the low ratio of infestation (7 : 18) in this series of experiments, as compared with the high ratio (7 : 9) in the former series, it may be said that the number of cercaria per crab was much smaller in the latter than in the former series.

In a third series of experiments *Emerita* were held from one to several days in jars[6] containing infested snails for comparison with crabs held in running sea water. The result of this experiment was an infestation of 17 out of 35 crabs in the experimental jars compared with 6 out of 46 in the control. The probability of this result on a purely chance basis is .0003.[7]

[4] Computed from McEwen (1929).

[5] In determining the presence or absence of parasites in the crabs the factor of length of examination is essential. I adopted a minimum of 12–15 minutes for the smaller (5–6 mm.) specimens and of 15–20 for the larger (10–15 mm.) ones.

[6] Some of these were supplied with running sea water. In others the water was standing, but aerated.

[7] Computed from McEwen (1929).

In evaluating this series of experiments it should be observed that in many of them only a very meagre number of cercariae were furnished a considerable number of crabs in a volume of from two to eight liters of water, hence the chance of any individual crab receiving a cercaria was rather small. The infestation of a considerable number of crabs in the controls (6 in 46) is difficult to explain. Very rarely cercariae occurred in the laboratory water supply, but straining through cotton a small stream of water from a tap for about forty hours produced no cercariæ, so that their chance occurrence in the control aquaria can hardly be explained thus. In many cases the control aquaria were standing on the same table with jars which contained possibly infested snails and it is possible that cercariæ from the latter may have infested the controls. In one case in particular a control jar of *Emerita* collected on August 17, 1937 contained recently infested crabs until August 28, during which time a jar of snails was standing on the same table; but straining the water from this jar through fine bolting cloth on this date failed to produce any cercariæ. On August 30 and September 1 after removal of this jar an examination of ten crabs from this control revealed no recent infestations.

The source of the occasional infestation of the controls therefore remains uncertain, but in any case the difference in the infestation ratios between experiments and controls in this series is highly significant.

Taken collectively the experiments give a result of 31 infestations out of 62 tests, while the controls give 11 out of 81 tests; and these include 5 out of 15 exposed to natural infestation on the beach. This result, on the basis of chance alone, has a probability of .0000013.[8]

Larvæ similar to those in the sand crabs occur in the fiddler crabs (*Uca crenulata*) from Mission Bay, an inlet of the sea near La Jolla. Doubtless, many other crabs of the vicinity harbor the same or related larvæ, for Lebour (*loc. cit.*) and Stunkard (*loc. cit.*) in Europe have found related metacercariæ in several species of crabs.

The extent of infestation of the willet and godwit by this parasite is rather limited. Only toward the end of this study did I differentiate between this species and two other related ones so that I cannot give any figures on the percentage of infestation. However, the former is usually, if not always, greatly outnumbered by what appear to be two other species of *Levinseniella*. In at least one case, that of a marbled godwit, it was apparently absent, but time did not permit an examination of the entire gut of this bird so that possibly a few specimens escaped observation.

[8] Computed from McEwen (1929).

McMullen (1935) gives some data which are of interest in this connection. In experimental infestations of *Amia calva* with *Macroderoides typicus* he found the fish heavily infested a few days after feeding infested material, but after several months the infestation was only slight. Thus one fish examined after 22 days contained by actual count at least 1392 flukes, while another fish examined 202 days after infestation contained only 33. He believes that "This enormous loss . . . was probably . . . due to crowding" (*loc. cit.*, p. 375). And Sarles and Stoll (1935, p. 290) state that "cats . . . carrying natural infestations of *Toxacara cati* were found to possess a uniformly high degree of resistance against attempts to superimpose infection with this ascarid." What limits the degree of infestation in wild animals, many of whom, as is the case with these shore birds, have unlimited opportunity for acquiring it, is as yet an unsolved problem. It may be, as suggested by Stunkard (1930) and Stoll (1929), analogous to the development of immunity by animals to bacterial infection. In the case of *L. cruzi* and the shore birds it is possible that the usually abundant infestations with other species limits the number of the former. It does not, however, apparently interfere with extensive infestation by a *Maritrema sp.* which the birds probably receive from the sand flea (*Orchestoides*), which forms a considerable part of their diet.

In trying to follow the life history of this worm I was at first misled by finding excysted individuals in the surf perches (*Embiotoca*, *Abeona*) after the latter had eaten sand crabs. It was only after performing several experiments that I discovered that the infestation of the fish is only temporary. It is rather remarkable, however, that a parasite of a homoiothermal animal should excyst and exist, even for a brief period in a poikilothermal form. My observations on this point are not solitary, however, for Linton (1928) reports *Levinseniella adunca* from a sanderling (*Crocethia alba*) which "appeared to be identical with a species found in the toad fish (*Opsanus tau*)" (*loc. cit.*, p. 21).

The last link in the life history of this worm is not yet made. In spite of repeated fecal examinations of both wild and captive birds, I have not succeeded in finding either eggs or miracidia. The eggs in utero appear never to have developed beyond an early cleavage stage. I have one probable record of a miracidium in the snail, but as my observations were very brief, the organism being lost in transfer to a slide, I am not able to assert positively that it *was* a miracidium, much less to determine whence it came. There must, however, be some period when the birds are shedding eggs or miracidia. This I hope subsequent study will reveal.

Summary

The life history of a trematode (*Levinseniella cruzi?*) from the shore birds (*Limosa* and *Catoptrophorus*) and the structure of its cercaria have been described in some detail. The probable life history is as follows:

The miracidium has not yet been certainly observed. The cercaria, which belongs to the ubiquita group of Lebour, inhabits the snail, *Olivella biplicata*. From here it passes to the sand crab, *Emerita analoga*, which latter, when eaten by the birds, infests them with the adult trematode. The degree of infestation of the snail is considerable, but the cercariæ are shed infrequently, possibly at definite seasons.

Practically all crabs over 6 to 7 mm. in length are infested, but for some as yet undetermined reason, the infestation of the birds is light.

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